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# The usage of mesenchymal stem cells in the treatment of type 1 diabetes mellitus

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# THE USAGE OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF TYPE 1 DIABETES MELLITUS

by

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**THE USAGE OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF TYPE 1 DIABETES MELLITUS**

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ABSTRACT

Type 1 Diabetes mellitus is a metabolic disorder characterized by an autoimmune attack against the insulin producing Beta-cells of the pancreas. Also known as insulin-dependent diabetes, patients must receive exogenous injections of insulin in order to maintain glycemic homeostasis. The necessity of monitoring one's own blood glucose levels and self-administering insulin is a tedious routine for type 1 diabetics, and this standard treatment option fails to treat any of the underlying causes of the disease. According to van Belle et al, the prevalence of diabetes is rising worldwide amongst all age-groups, from 2.8% in 2000 to an estimated 4.4% by 2030, thus the need to find a more curative treatment approach is eminent. In the emerging field of regenerative medicine, mesenchymal stem cells have been identified as a possible therapeutic tool to replace damaged parenchymal tissue. Along with their ability to modulate the local microenvironment, the introduction of properly differentiated mesenchymal stem cells into patients with Type 1 diabetes may provide a treatment option that helps supplement the lost islet cells without provoking an immune response. Preliminary clinical trials have shown that stem cell therapy decreases the amount of exogenous insulin required daily, decreases fasting glucose levels, decreases amount of glycated hemoglobin and increases C-peptide levels. These four indicators of diabetic control suggest that

mesenchymal stem cells are an effective means of helping manage Type 1 diabetes. Still, much research needs to be done to fully understand the biomechanics behind the cells' actions in order to expand human clinical trials. Although complete insulin independence is rarely achieved in patients receiving mesenchymal stem cell treatment, the promising results shown so far suggest more studies be undertaken in hopes of finding a corrective approach to treat Type 1 diabetes.

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AD-MSC…… Adipose derived mesenchymal stem cell

ASC……Adult stem cell

BM-MSC…….. Bone marrow derived mesenchymal stem cell

CTL…….. Cytotoxic T lymphocyte

ESC……Embryonic stem cell

GFP…… Green fluorescent protein

GM-CSF…….. Granulocyte-macrophage colony stimulating factor

HLA…….. Human leuokocyte antigen

HSC…….. Hematopoietic stem cell

IL4…….. Interleukin 4

IPC…….. Insulin producing cell

MHC……..Major histocompatibility complex

MSC……...Mesenchymal stem cell

PD-L1……..Programmed death ligand 1

T1DM ..... Type 1 diabetes mellitus

WJ-MSCs……..Wharton jelly derived mesenchymal stem cell

# INTRODUCTION

## Type 1 Diabetes Mellitus

Type 1 Diabetes mellitus (T1DM) is clinically associated with autoimmune damage to the pancreas, resulting in an inability to produce an adequate amount of insulin, but the root cause of the disease remains unknown. Patients typically present to a physician during childhood or early adolescence with symptoms caused by the high blood glucose (hyperglycemic) condition such as increased thirst, frequent urination, increased hunger, and weight loss. With T1DM, the body's own immune system targets and destroys the insulin producing Beta-cells within the Islets of Langerhans in the pancreas. The other subgroup of diabetes, Type 2 Diabetes mellitus, results from an inability of the body to properly respond to already secreted insulin. In the United States alone, over 1.25 million people are living with T1DM, and the prevalence of T1DM in people under the age of 20 has risen 20% over the last ten years[37].

Although the complete etiology of T1DM is not understood, there is believed to be an interplay between both genetic and environmental factors that cause a T-cell mediated destruction of the Beta-cells[1]. Autoantibodies that target the islets begin to appear months to years before the symptoms and can be used as biomarkers for T1DM. Individuals with particular human leukocyte antigen (HLA) gene complexes such as HLA-DQA1, HLA-DQB1, and HLA-DRB1 have a higher risk of developing islet-targeting autoantibodies. The HLA system is responsible for encoding the MHC proteins, whose cell-surface location plays an important

regulatory role in the immune system, and thus can be used to screen and study individuals who are at an increased risk of developing T1DM[50]. The first appearance and the abundance of these autoantibodies, along with the progression of Beta-cell destruction, underlay the continuum of the pathogenesis of T1DM, seen in Figure 1. Staging of T1DM is particularly useful when enrolling individuals in secondary prevention trials that aim to reduce the impact of a disease once an individual has been diagnosed[33].

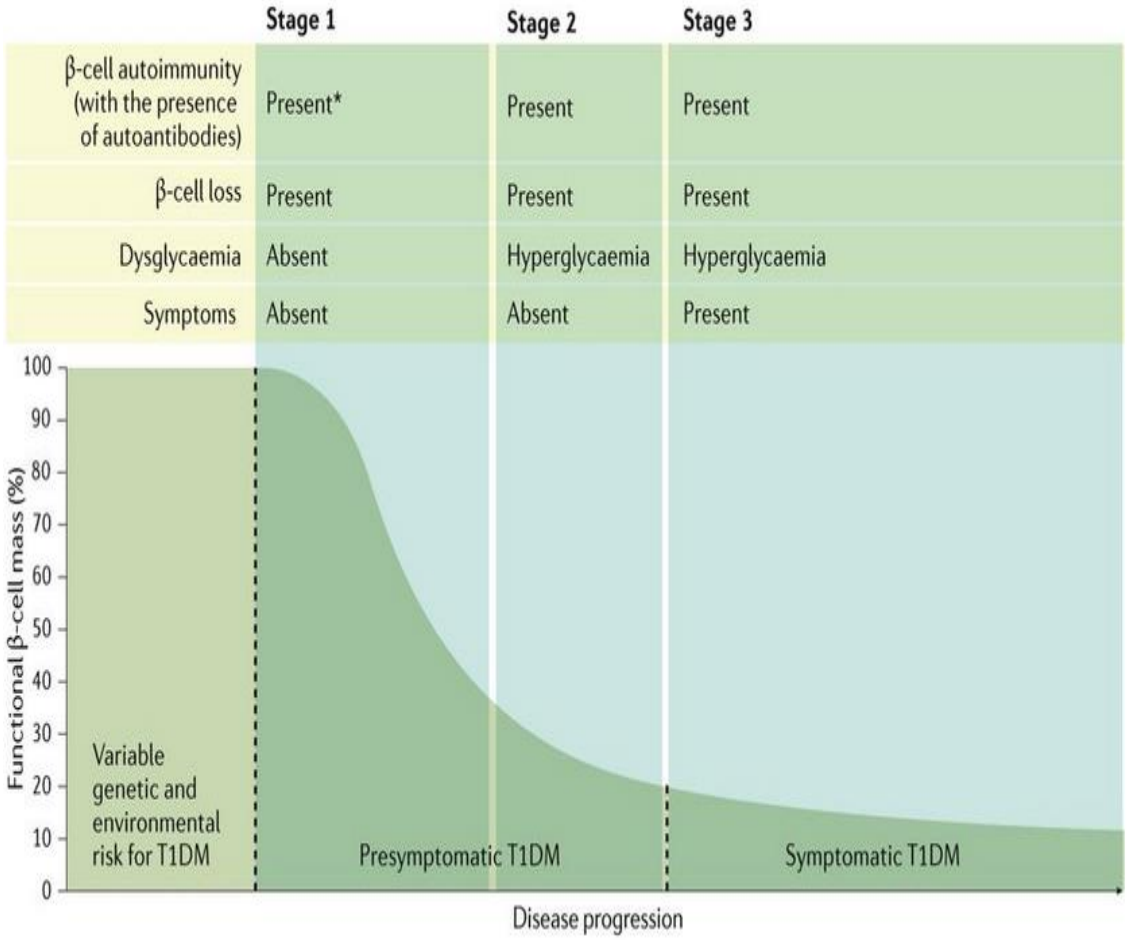


**Figure 1. Stages of T1DM:** Patients are categorized into 1 of 3 groups based on a variety of symptoms and physiological measures. *Beta-cell targeting autoantibodies may be present months to years before the onset of Beta-cell loss[33].

The appearance of the first islet-targeting autoantibody coincides with dendritic cells presenting the Beta-cell autoantigen and the subsequent responses of $CD^{4+}$ helper T cells and $CD^{8+}$ cytotoxic T cells to the autoantigen (Figure 2). B cell exposure to these Beta-cell autoantigens leads to the production of the islet-targeting autoantibodies, and antigen presentation by dendritic cells and other B cells drives the activation of Beta-cell specific T cells which attack the pancreas. Patients that have progressed to stage 3 T1DM have detectable levels of both $CD4^{+}$ and $CD^{8+}$ Beta-cell autoantigen specific T cells[46]. Insulin secretion decreases in parallel with the destruction of the pancreatic Beta-cells, eventually resulting in hyperglycemia as glucose fails to be properly absorbed and remains in the bloodstream. Acute symptoms such as polydipsia, polyuria, and polyphagia develop rapidly. Long-term systemic complications such as atherosclerosis, peripheral vascular disease, and an increased risk of infection can develop over time in individuals with diabetes[3]. Patients are also at risk for microvascular complications such as retinopathy, renal disease, and neuropathy, along with macrovascular complications such as stroke, heart disease, and hypertension. As a result of the autoimmune attack on the pancreas, diabetics have an increased risk of developing acute and chronic pancreatitis, inflammation of the pancreas, that in turn leads to an increased risk of pancreatic cancer[51].

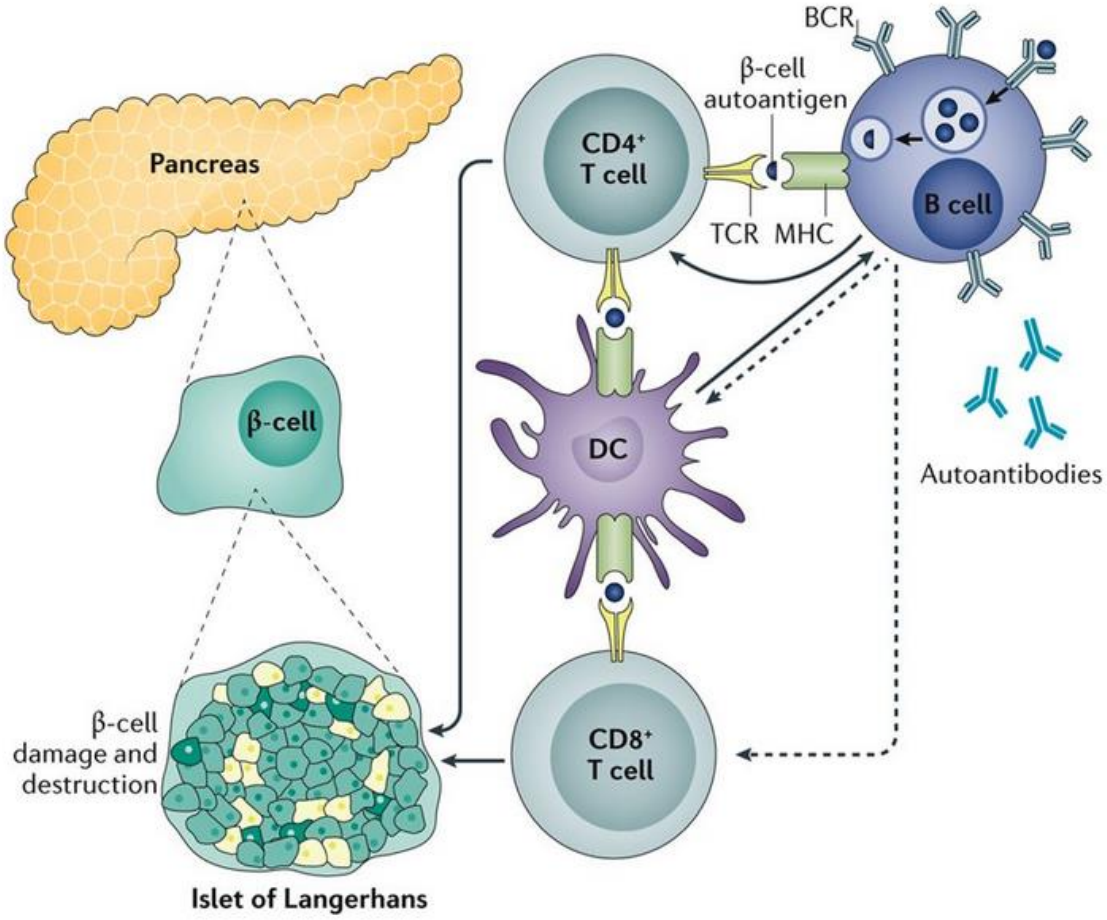


**Figure 2. Pathogenesis of T1DM:** The production of islet-targeting autoantibodies act as biomarkers indicative of the presymptomatic stage of diabetes. Dashed arrows show potential interactions between B cells and dendritic cells, and B cells and $CD^{8+}$ T cells[33].

Management of T1DM primarily aims at glycemic control and the promotion of a healthy lifestyle in order to prevent severe hyperglycemia and ketoacidosis. To date, insulin therapy remains the most widely used treatment option, with the daily introduction of exogenous insulin dosed based on body weight, gender, blood glucose levels, and other considerations[10]. Other less commonly used treatment options include amylin analogues, whole pancreas or islet transplantation, as well as

stem cell therapy. None of these treatment options have proven completely curative, and all have flaws. Insulin treatment requires the patient to constantly monitor his or her glucose levels by taking blood samples via prick and measuring them via a blood glucose monitoring device and then administer the proper amount of insulin as needed. Amylin analogues act synergistically with insulin and is only given as a supplement rather than as a stand-alone treatment. Pancreas and islet transplantation is limited by the number of donors and reserved only for severe cases of T1DM due to the requirement of lifelong immunosuppression usage associated with such transplants. Still, these alternative treatments have shown the ability to reduce the severity of hyperglycemia and have been prime targets for further experimentation[6]. Recent advancements have led to the ability to differentiate mesenchymal stem cells (MSCs) into insulin-producing cells (IPCs) in a laboratory environment, yet little is known about long term efficacy of such treatment *in vivo*. With a doubling time between 48-72 hours and the capability to undergo more than 60 rounds of doubling, obtaining a large enough sample of MSCs for expanded human clinical trials is becoming readily available[12].

**Mesenchymal Stem Cells**

The shortcomings mentioned in the current treatments for T1DM has led to the development of more novel therapeutic approaches. The current standard for the treatment of T1DM, exogenous insulin injections, fails to adequately mimic the activity of endogenous insulin when administered over many years and requires the patient to maintain a strict routine for monitoring his or her own blood glucose

levels. With this treatment, the damage to the Beta-cells continues to worsen as the autoimmune attack on the pancreas is not interfered with. As the therapeutic potential of stem cells has started to take hold in other fields of regenerative medicine, their usage in the treatment of diabetes is starting to be explored as a means of treating the underlying causes of diabetes rather than solely focusing on managing the symptoms[57].

After being administered, stem cells are able to migrate to sites of injury via a chemoattraction process utilizing integrin proteins, and once at the site are able to undergo further, more specialized, differentiation which can replace damaged tissues[20]. These cells are classified into two groups based on their origin: embryonic stem cells (ESCs) or adult stem cells (ASCs). ESCs are pluripotent and can differentiate into any germ line cell, however they are scarcely used in clinically due to the ethical controversy surrounding their harvesting process. Instead, ASCs, which are multipotent, and thus can differentiate into fewer cell types than ESCs, are the current model of stem cell being explored for therapeutic properties. ASCs are further divided into hematopoietic stem cells (HSCs) and MSCs. MSCs have been noted to serve an immunosuppressive role in transplantation by inhibiting T-cell and B-cell proliferation, and can be genetically modified by adding the desired MHC complex so as to reduce the chance of an immune rejection[40].

The usage of MSCs in diabetes treatment has been explored due to their capability to generate and support existing insulin producing Beta-cells within the pancreas, and thus they provide a novel and exciting therapeutic tool for the

treatment of T1DM. Initially, bone marrow derived MSCs (BM-MSC) were the most common source of MSCs used in the clinic, as bone marrow provides the largest reservoir of both MSCs and HSCs[42]. Isolation of stem cells from bone marrow relies on the physical adherence of the cells to the plastic cell culture plate used in the isolation and purification process of the human bone marrow sample. Flow cytometry is then used to identify specific antigen expression of CD factors that are expressed exclusively on MSCs, and then histology staining is used to indicate the MSCs are capable of proper lineage differentiation. Due to the invasive and painful procedure required to collect the MSCs from the human sample, this source has decreased in popularity as other sources of stem cells have become more accessible and efficacious[5].

Over the last decade, the usage of adipose tissue as a source of MSCs has been increasingly used by researchers due to its abundance of cells and easily accessible subcutaneous location. In this procedure, small pieces of excised fat are washed and explanted to tissue culture flasks where they incubate for 3-5 days in proper medium. A similar differentiation procedure as the BM-MSCs is then used to ensure properly functioning adipose derived mesenchymal stem cells (AD-MSCs) are isolated[24].

A direct comparison between AD-MSCs and BM-MSCs has revealed in multiple studies that AD-MSCs have greater proliferative, differentiation, and immunomodulatory potential. In one such study, ischemia in mice was induced by ligating the femoral artery, and the animals were treated with either BM-MSCs, AD-

MSCs, or a control. 14 days post-transplantation, the mice treated with AD-MSCs showed a greater proportion of perfusion in the ischemic area when compared to the mice treated with BM-MSCs (Figure 3A). The skeletal muscles of the mice who received AD-MSCs had a lower relative area of injured tissue when compared to the mice receiving BM-MSCs (Figure 3B). The greater efficacy of AD-MSCs combined with the simpler harvesting process than BM-MSCs have made AD-MSCs the mostly widely used stem cell source in regenerative medicine[35].

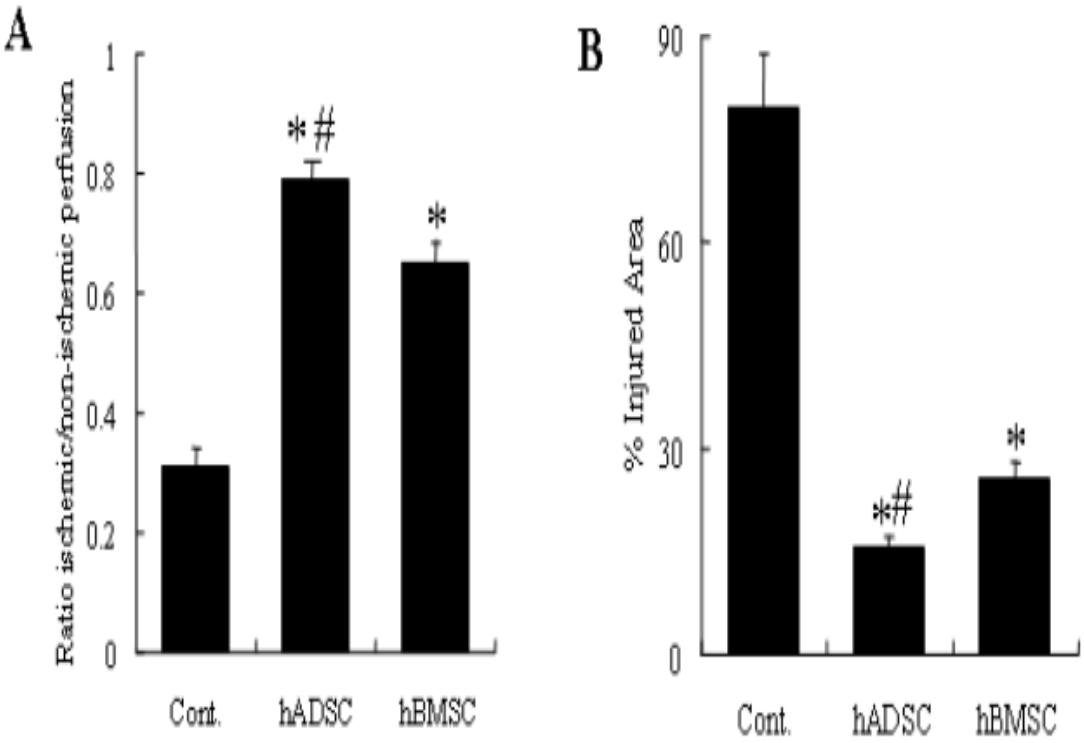


**Figure 3. Comparison of AD-MSCs and BM-MSCs:** A) The amount of ischemic perfusion in the ligated tissue was grater in mice that received AD-MSCs. B) Mice receiving AD-MSCs showed less injured tissue 14 days post-transplantation compared to mice receiving BM-MSCs. *signify data that are significantly different from control group; # signify data that are significantly different from BM-MSC group[35].

An even more recent approach to harvesting MSCs has been via a non-invasive mechanism by isolating them from the placenta or umbilical cord. Stem cells derived in this manner have shown similar proliferative and differentiation abilities and an increased ability to express paracrine factors that are needed by insulin releasing cells when compared to BM-MSCs[48]. Wharton's jelly (umbilical cord matrix) is an especially abundant source of MSCs and isolation is associated with higher yield and comparable percentage of successful differentiation compared to other sources[44]. The two most common methods for isolation of MSCs from Wharton's jelly are shown in Figure 4. The enzymatic method involves the usage of enzymes that disrupts the collagen matrix and releases cells into another solution that can then be centrifuged and plated on a tissue culture dish with stem cell media. The explant method involves direct transfer of umbilical cord tissue fragments onto a tissue culture dish that is filled with media that promotes the propagation of the stem cells[44]. Nevertheless, this source of stem cells is limited by the number of available donors.

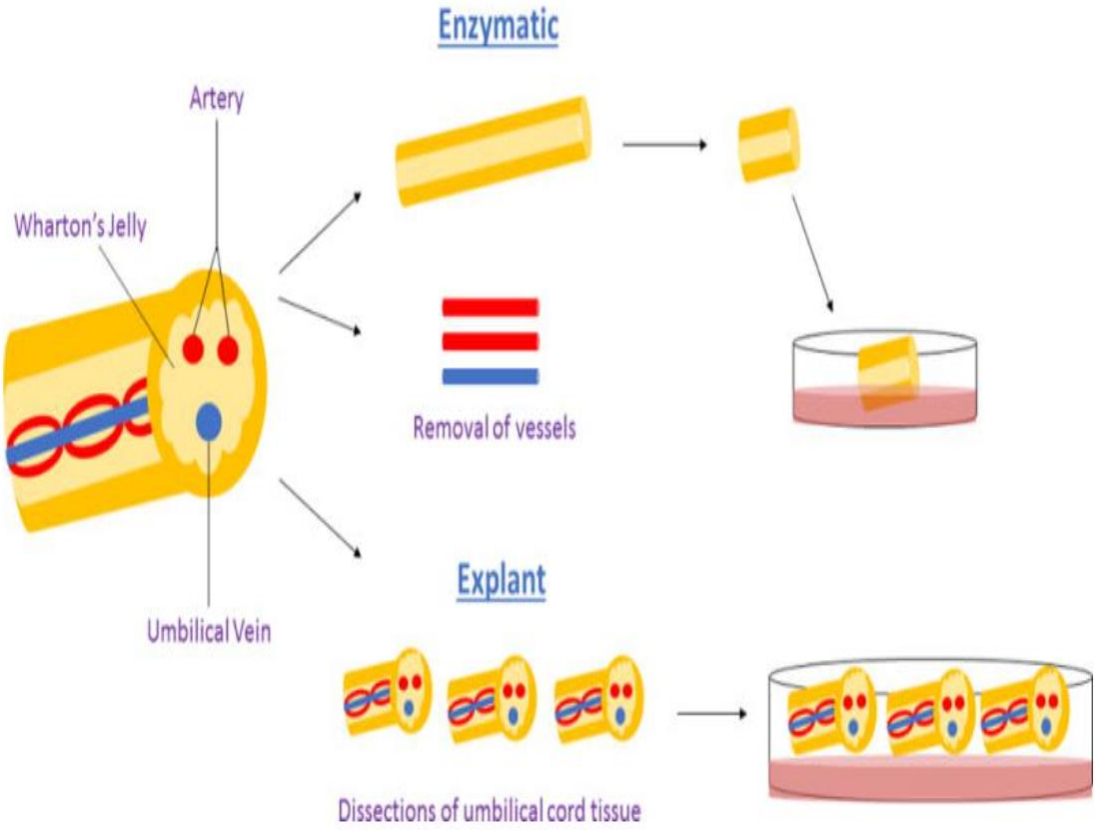


**Figure 4. Enzymatic vs explant method for obtaining MSCS from Wharton's jelly:** MSCs are able to be obtained from the umbilical cord matrix via an enzymatic process that digests the tissue and leaves individual cells that can be harvested on a growth plate. The explant method is a simpler process because it requires less manipulation of umbilical cord tissue[44].

Both adipose tissue and bone marrow can provide a source of autologous MSCs, where the source of MSCs is from the individual himself. The bone marrow isolation process yields higher numbers of MSCs when compare to adipose tissue, but the difficulty in recruiting donors has limited the number available for research purposes. Further research has indicated that BM-MSCs may also suffer from contamination by HSCs, endothelial cells, and progenitor cells which may lead to a

heterogeneous mixture of cells. Allogenic sources of stem cells such as Wharton's jelly or umbilical cord matrix are used when the patient is too ill to have their own tissues isolated or when there is not enough time to allow for the expansion and differentiation process to occur[52].

Individuals with T1DM retain their own functional MSCs that can be autogenically transplanted to initiate endogenous pancreatic regeneration. Further, BM-MSCs taken from Type 1 diabetic patients have shown the ability to differentiate into IPCs under suitable conditions[55]. This would permit the patient to use his or her own stem cells as a source of IPCs, thus bypassing the need for a donor. Although feasible, chronic hyperglycemia has been shown to promote a defective microenvironment in diabetics that impair proper MSC functions by diminishing their reparative capabilities as well as reducing their migratory and proliferative potential[14]. Thus, a healthy, third-party source of MSCs seems to be of great necessity for stem cell transplantation in diabetics, as MSCs play an active role in the healing process of damaged tissues by replacing dead cells and secreting factors that activate surrounding cells that enhance tissue repair[55]. Therefore, it has been theorized that MSC transplantation can be used to treat tissues impaired by chronic hyperglycemia by increasing Beta-cell mass. This is accomplished via Beta-cell replacement through *in vivo* or *in vitro* differentiation, production of cytokines and other local microenvironment factors that stimulate the regeneration of endogenous MSCs, and reducing the autoimmunity to Beta-cells[21].

The impressive safety record in clinical trials has made MSCs a promising candidate for future studies in regenerative medicine. Donor MSCs must be mobilized into the peripheral target (pancreas) for the regeneration of Beta-cells. The goal is to provide a sufficient number of MSCs to the pancreas that might differentiate into islet cells and also activate endogenous stem cells by providing a suitable microenvironment[27]. Two methods are used in the transplantation process: intravenous injection and direct injection. The intravenous method is the most convenient method for stem cell transplants, but in diabetes, the intrapancreatic transplantation method is ideally used due to the higher therapeutic potential, with the goal of the transplanted MSCs being also to prime endogenous stem cells so the two lineages can jointly repair the damaged tissue[39]. To ensure homogeneity throughout the pancreas, up to ten injection sites are selected. Intravenous injection is used in situations when the pancreas is not easily accessible in the patient, as injected cells become sequestered in the lungs, therefore limiting the number of effective MSCs reaching the target site[22]. Figure 5 provides an overview of the physiological consequences of MSC transplantation in diabetic patients.

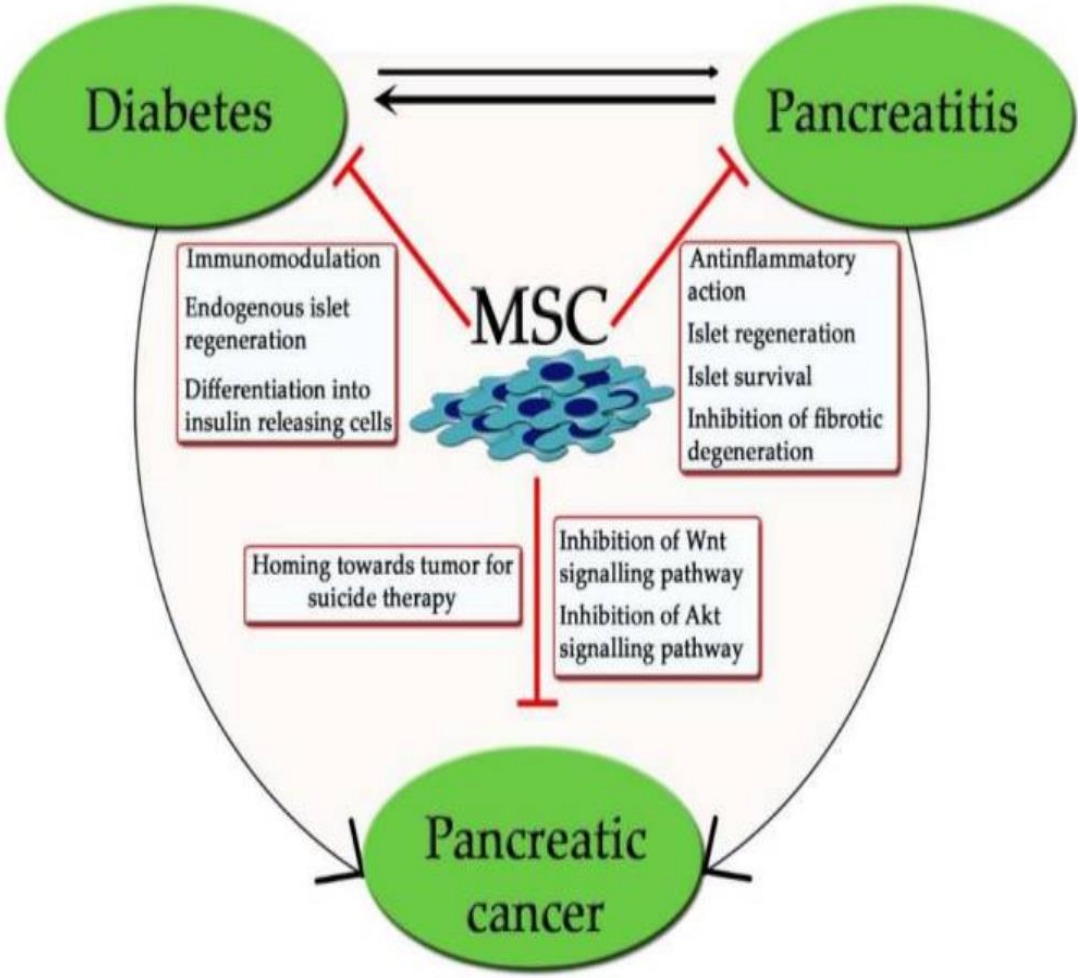


**Figure 5. MSC mechanisms on pancreatic disorders:** The immunomodulatory and pro-survival properties of MSCs allow them to be used to prevent disease progression by protecting the pancreas and relieving disease symptoms[51].

Although this paper focuses on how MSCs are currently being applied in diabetes treatment, they are not the only type of stem cell that may possess applicable therapeutic benefits. HSCs are defined by their ability to self-regenerate as well as differentiate into all mature blood cell types. These include cells of the lymphoid lineage such as B cells, T cells, and NK cells, as well as cells of the myeloid lineage such as macrophages, granulocytes, megakaryocytes, and erythrocytes. HSC

usage for transplantation purposes has been investigated due to the observed relationship between hematopoiesis and vasculogenesis. A constant flux of HSCs migrating from either blood to bone marrow (homing) or from bone marrow to blood (mobilizing) allow for redistribution throughout the entire circulatory system[60]. Along with providing an immediate source of progenitor cells in cases of blood loss or cellular damage, HSCs have been shown to contribute to the repair of chronically injured tissues that are nonhematopoietic in nature[36]. In the face of selective pressure, such as damaged tissue, HSCs may be recruited in an atypical fashion leading the nonhematopoietic cell outcomes. Whether this occurs by transdifferentiation or by cell fusion with endogenous progenitors, remains unknown[61]. This unique property of HSCs has sparked more research into the biochemical mechanisms underlying this change of function, and has led a rise in the usage of HSCs in the field of regenerative medicine.

The physiological potential of HSCs in treating T1DM alone warrants them a greater focus in explorative research. When it was discovered that hyperglycemia can induce abnormal gene expression in both HSCs and their progeny, their role in the disease became more complicated. Peripheral diabetic neuropathy is one of the major complications of chronic diabetes, damaging nerve cells and impairing the microvascular vessels that supply the nerves, often seen in the legs and feet. This, in turn, causes a change in the microenvironment of the bone marrow. Consequently, a consistent hyperglycemic environment can directly affect HSCs, leading to fusion of their progeny with dorsal root ganglion neurons, causing the production of TNF-

α, a cytokine that has been implicated in the development of insulin resistance, along with apoptosis of the HSCs themselves. Even in response to normoglycemic control or antioxidants, the pathogenesis of diabetic neuropathy does not completely remit and the altered bone marrow phenotype is preserved[32]. Thus, the loss of properly functioning HSCs is a concern for diabetic patients, and treatment via exogenous stem cell transplantation may prevent further insulin resistance in Type 1 diabetics who are already struggling to produce adequate amounts of insulin.

**Goals**

As it is such a developing field, most of the published literature regarding the safety and efficacy of MSCs are from studies using animal models. The objective of the thesis is to provide the reader with a condensed overview of current human trials being undertaken that involve type 1 diabetics treated with MSCs. In doing so, potential highlights of the field will be illuminated while also providing an overview of current limitations. MSCs have already shown regenerative capabilities *in vivo*, thus their usage in supplementing damaged islet cells has an exciting future. Much has recently been discovered about the mechanism of action MSCs undertake in the pancreas as well as the efficacy of such treatment. This thesis will examine the physiological processes that occur *in vivo* that make MSCs an attractive alternative treatment option for T1DM. Exogenous insulin remains the gold standard treatment, but its popularity is not rooted in its capability to treat the disease, but rather in its capability in managing the disease. This paper aims to explore how

MSCs may provide a therapeutic outlet that manages the underlying autoimmune destruction of pancreatic Beta-cells. By providing a clear and concise overview of how such stem cells can produce therapeutic benefits for those with T1DM, this thesis can serve as a reference for patients considering a promising alternative treatment rather than the standard insulin doses.

## PUBLISHED STUDIES

### Immunomodulatory Properties of MSCs

The capability of MSCs to escape immune recognition along with their immunomodulatory potential have led to their reception of great interest in transplantation and regenerative medicine. When these functions were first noted, the exact underlying mechanisms could not be fully explained, which prompted further experimentation on MSCs in diabetic mice models. Multiple *in vitro* and *in vivo* studies have illustrated the ability of MSCs to impair proliferative activities and functions of B and T cells via inhibition of cyclin expression[25]. Production of soluble factors such as Prostaglandin E2,, and interleukin-10 arrest B and T cells in the cell cycle and hinder replication[19]. MSCs are also capable of inhibiting differentiation, maturation, and function of dendritic cells, the most important antigen-presenting cells[31]. Importantly, MSCs are not recognized by natural killer cells and can avoid recognition by T-cells, thus potentiating their survival *in vivo*[49].

Dendritic cells are involved early on in the immune process. They are derived from a $CD14^+$ monocyte precursor, where MSCs play an important modulatory role. Jiang et al investigated the effects of human MSCs on the function, differentiation, and maturation of monocyte derived dendritic cells *in vitro*. A MSC culture induced with granulocyte-macrophage colony stimulating factor (GM-CSF) plus interleukin 4 (IL4) was shown to strongly and reversibly inhibit the initial differentiation of monocytes into dendritic cells. Figure 6 shows monocytes cultured in the presence of GM-CSF and IL-4 and monocytes treated with MSC culture.

Mature dendritic cells treated with MSCs were shown to have reduced expression of CD1a, indicating a transition back to immature status[31].

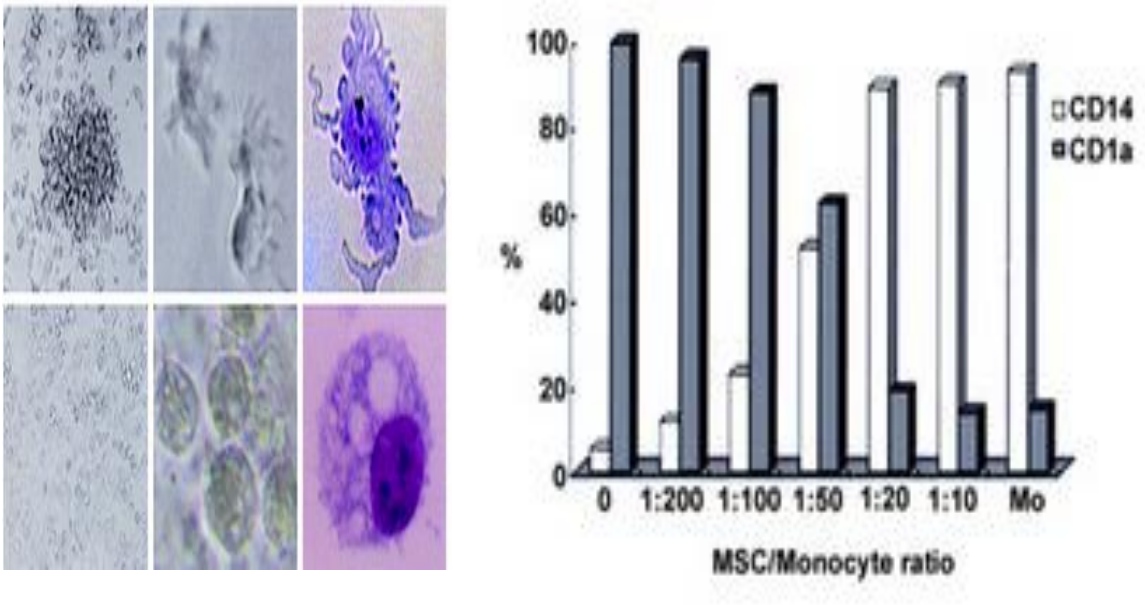


**Figure 6. MSCs inhibit the initial differentiation of monocytes into dendritic cells:** (Left) Monocytes cultured with GM-CSF and IL4 resemble mature dendritic cells (top), whereas monocytes cultured with MSCs display macrophage-like morphology (bottom) when examined by phase contrast microscopy. For the left, middle, and right columns for both rows: original magnifications x40, x200, and x1000, respectively. (Right) CD1a and CD14 expression of monocyte-derived cells in MSC/monocyte cultures at differing ratios[31].

When studied *in vitro*, it has been shown that MSCs naturally synthesize growth factors and cytokines that are primarily influenced by the local microenvironments that the MSCs are in. Several of these trophic factors promote the survival of surrounding cells and also play an important role in the regulatory properties of the MSCs themselves[2]. Thus, MSCs transplanted into the pancreas will

produce pro-survival cytokines that can act on the endogenous Beta-cells. Furthermore, MSCs have been shown to release microvesicles into the extracellular space through budding of the plasma membrane, which is important for cell to cell communication[43]. Microvesicles produced by MSCs have similar surface characteristics (such as similar CD factors) and contain biologically active molecules that can be transferred to recipient cells via internalization or membrane fusion. Once in the target cell, microvesicles are able to alter transcription, proliferation, and immunoregulation, thus leading to functional changes of the target cell. In one study, co culturing MSCs with microvesicles in patients with T1DM lead to an increase in regulatory T cell counts as well as helped induce a more immunological tolerant phenotype[43]. For type 1 diabetics, these microvesicles may play a role in deterring the autoimmune Beta-cell destruction.

To elucidate the mechanism by which MSCs modulate T cell count, Rasmusson et al. took a collection of cytotoxic T lymphocytes (CTL) that were primed and activated against stimulator lymphocytes and added MSCs to a solution. Both CTLs and natural killer cells function to kill pathogen-infected or neoplastic cells, but natural killer cells work as part of the innate immune system while CTLs recognize antigens, are cell-mediated, and are more specific. The cytotoxic potential of the CTLs were analyzed in a chromium-release assay at various time points after the addition of the MSCs, and compared to a CTL culture that did not receive MSCs. A similar experiment was conducted with natural killer cells, where the natural killer cells were mixed with MSCs and the lysis was measured[49]. When added at the

beginning of the culture, the MSCs were shown to inhibit CTL-mediated lysis by 70%, but the lysis was unaffected when in the cytotoxic phase (days 3-6). This indicates that MSCs act to inhibit CTLs early in the activation process, since little to no cytotoxicity was reported when MSCs were added after day 3[49]. A later study revealed that MSCs appear to impair T-cell proliferation via inhibition of cyclin expression, which arrests T cells in the G0/G1 phase of the cell cycle[28]. In addition, it was shown that the MSCs were able to inhibit the formation of cytotoxic lymphocytes when the cells were separated via a transwell system, indicating that the MSCs act via a soluble factor. The MSCs were not lysed by either CTLs or natural killer cells, thus demonstrating their ability to escape recognition[39]. Alongside suppression of T cells, MSCs have also been noted to suppress the proliferation of stimulated B cells at the same cyclin checkpoints. Inhibition occurs in a dose-dependent manner, but at very low dose stimulating effects were observed[17].

An immunomodulatory examination of the surface of MSCs detected an intermediate level of MHC class I molecules on their surface. The expression of particular surface adhesion molecules such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 along with a number of integrin proteins allow for the direct interaction with T cells[38]. No MHC class II molecules or other costimulatory CD molecules were expressed, contributing to the cells avoidance from T cells. Such suppression of T cell activity occurred regardless of the source of the MSCs[38]. There have been supplemental studies showing that MSCs increase the amount of regulatory T cells while suppressing the function of other T cell

populations[28]. All together, these properties of MSCs demonstrate their durability *in vivo*. After being transdifferentiated into IPCs and transplanted, these cells can avoid the autoimmune damage that plagues the endogenous Beta-cells in T1DM.

These immunomodulatory properties of MSCs has certainly made them an attractive choice for research in regenerative medicine. The first reported IPC generated from stem cell differentiation came in 2001 using ESCs[4]. However, ESCs form teratomas, are surrounded by ethical controversies, and are limited in number. *In vitro*, MSCs have shown the capacity to differentiate into several cell lineages, including IPCs[41]. When cultured under specific conditions, MSCs that have been precommitted to one lineage can differentiate into other cell types via a process known as transdifferentiation[34]. These precommitted cells are able to respond to extracellular clues and dedifferentiate into a more primitive stem cell or are able to act as a cellular vehicle for the expression of the human insulin gene[54]. A general overview of the transdifferentation process for IPCs is presented in Figure 7.

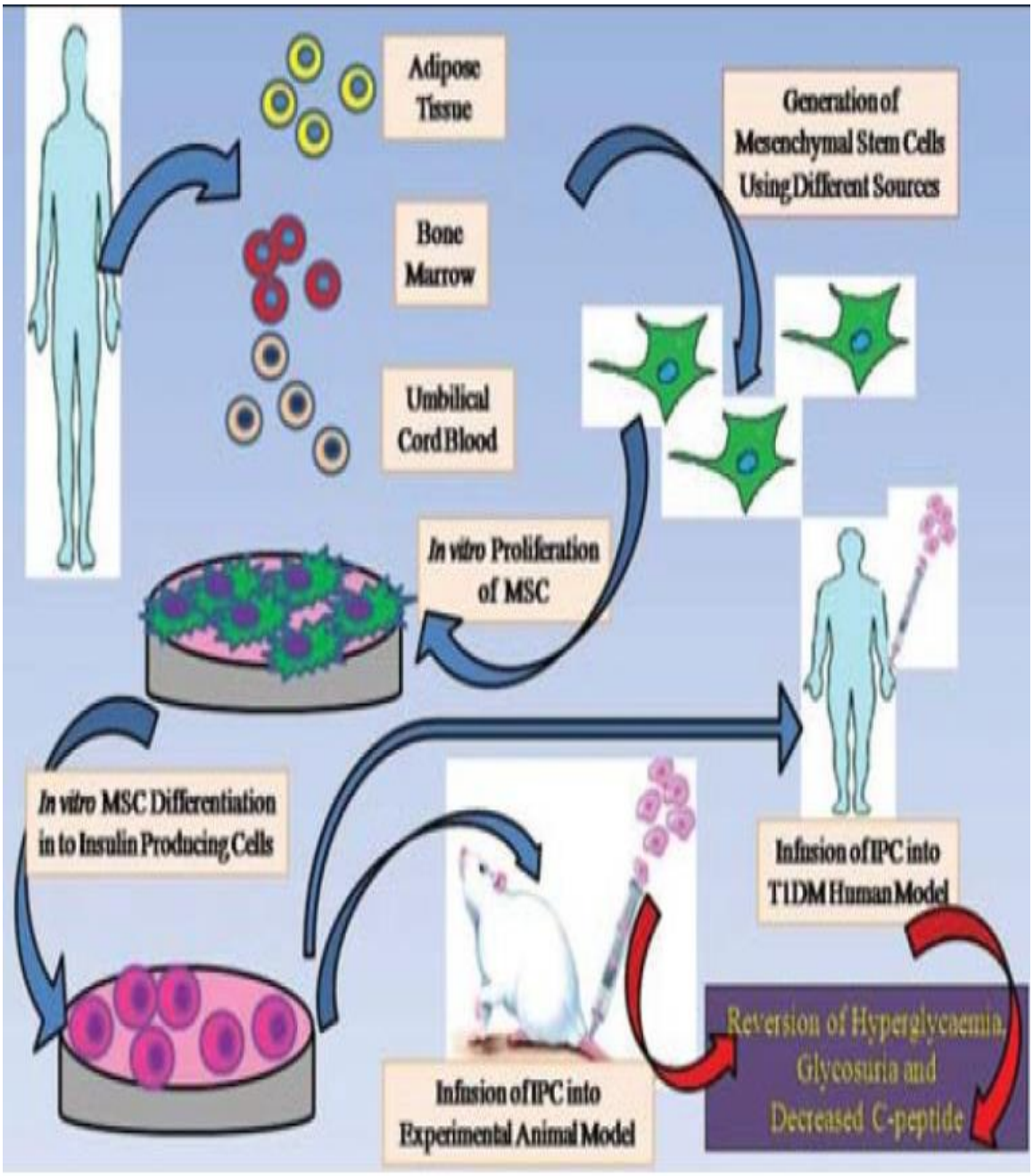


**Figure 7. *In vitro* transdifferentation of IPCs from MSCs:** Schematic representation of the various sources of MSCs and the differentiation procedure into IPC for transplantation[18].

Two distinctive pathways exist for obtaining functional Beta-cells from MSCs: gene-reprogramming and factor-based transdifferentiation. Gene-therapy involves the transfer a foreign gene into the MSC that activates or represses the insulin gene on demand. The factor-based approach involves surrounding the MSCs in specific culture conditions containing extrinsic insulin-promoting factors and cytokines *in vitro* culture[23]. A careful stage-wise addition and subtraction of a combination of extrinsic insulin-promoting factors is required for successful transdifferentiation. These biologically active factors are used endogenously in the endocrine pancreas differentiation, promote Beta-cell proliferation and differentiation, as well as increase insulin content of known IPCs[54]. Commonly used insulin-promoting factors and their roles in the pancreas are summarized in Table 1. Successfully differentiated MSCs are identified via an established IPC identification process. Cells are selected based on their expression of particular genes associated with pancreatic development such as *GLUT2*, *IslI,* and glucose kinase, as well as the identification of C-peptide, indicating successful endogenous production of insulin. C-peptide is a short amino acid chain in the proinsulin molecule that gets cleaved, but is not present in insulin given to diabetics[13]. Finally, the transdifferentiated IPCs are transplanted into the receiving individual[18].

| Extrinsic factors | Role |
|---|---|
| Nutrients and other factors | |
| High glucose | Increases β-cell replication and hypertrophy besides neogenesis |
| Amino acids | Indirectly affect β-cell proliferation through |
| N2 and B27 serum supplements | Acts as a serum supplement in serum-free mediums |
| Nicotinamide | Associated with the development of β-cell outgrowths from undifferentiated epithelial cell clusters |
| Growth factors, cytokines and hormones | |
| Activin A and betacellulin | Promotes β-cell regeneration by increasing β-cell mass |
| Epidermal growth factor | Accelerates high degree of B-cell proliferation and high degree differentiation |
| Pentagastrin | Markedly expands β-cell mass, specifically when it is combined with other factors |
| Glucagon-like peptide-1 and exendin 4 | Accelerates functional maturation of fetal β-cells as evidenced by their glucose-stimulated insulin secretion |
| Hepatocyte growth factor | Functions as an insulinotropic factor |

**Table 1. The role of various extrinsic factors in promoting IPC differentiation:** A wide variety of extrinsic factors are involved in the promotion of IPC differentiation and insulin production[18].

Transplantation of MSCs has proven to be useful in slowing the progression of diabetes, as intravenously or intrapancreatically administered MSCs are capable of recovering endogenous islets as well as reverting hyperglycemia[21]. The traditional view of stem cells would credit these therapeutic benefits to MSCs acquiring the phenotype of the parenchymal cells and replacing the dead cells. Yet, the published studies that have examined the number of transplanted MSCs that functionally integrate into damaged tissue have found the levels too low to support such a physiological change[47]. Therefore, the main contribution of MSCs in tissue regeneration may primarily lay in their immunomodulatory properties and ability to boost the function of surviving endogenous Beta-cells. MSCs can limit T lymphocyte activity and stimulate regulatory T cells, as well as secrete anti-inflammatory cytokines[59]. In autoimmune animal models, administered MSCs home into the damaged organ and prevent the destruction of newly formed and remaining endogenous cells, produce trophic factors that prevent the apoptosis of remnant cells, and induce the proliferation and differentiation of local progenitor cells[8].

Ezquer et al attempted to elucidate the mechanism underlying some of the antidiabetic effects of MSCs. First, he attempted to assess whether transplanted MSCs with green fluorescent protein (GFP) differentiated into Beta-cells and/or modified pancreatic and systemic physiological markers of T1DM in mice. As anticipated, no cells were detected that expressed both insulin and GFP in the recipient mice's pancreas, indicating that administered MSCs do not differentiate *in vivo* into IPCs. Rather, the donor MSCs were seen in secondary lymphoid organs,

where they inhibit the proliferation of the autoaggressive T cells by either modifying local cytokines or inducing more regulatory T cells. The distribution of MSCs-GFP as well as presence of pancreatic cells expressing both insulin and GFP was determined via immunohistofluorescence and flow cytometry[21].

In addition, the abundance and functionality of regulatory and autoaggressive T cells was measured via flow cytometry, and both the pancreatic and systemic expression of both anti-inflammatory and proinflammatory markers along with intraislet apoptosis was recorded. The autoaggressive T cells are responsible for a pro-inflammatory response, stimulating B cells to produce antibodies, and attacking the Beta-cells directly. Both seven and 65 days after the transplantation, the mice treated with MSCs showed significantly lower amounts of autoaggressive T cells in both the pancreatic lymph nodes (local) and spleen (systemic), along with an increase in regulatory T cells in both locations. Proinflammatory cytokine production (when stimulated *ex vivo*) was lower in the MSC receiving mice as well[22]. Comparisons between the untreated diabetic mice and mice receiving the MSC transplantation is presented in Figure 8.

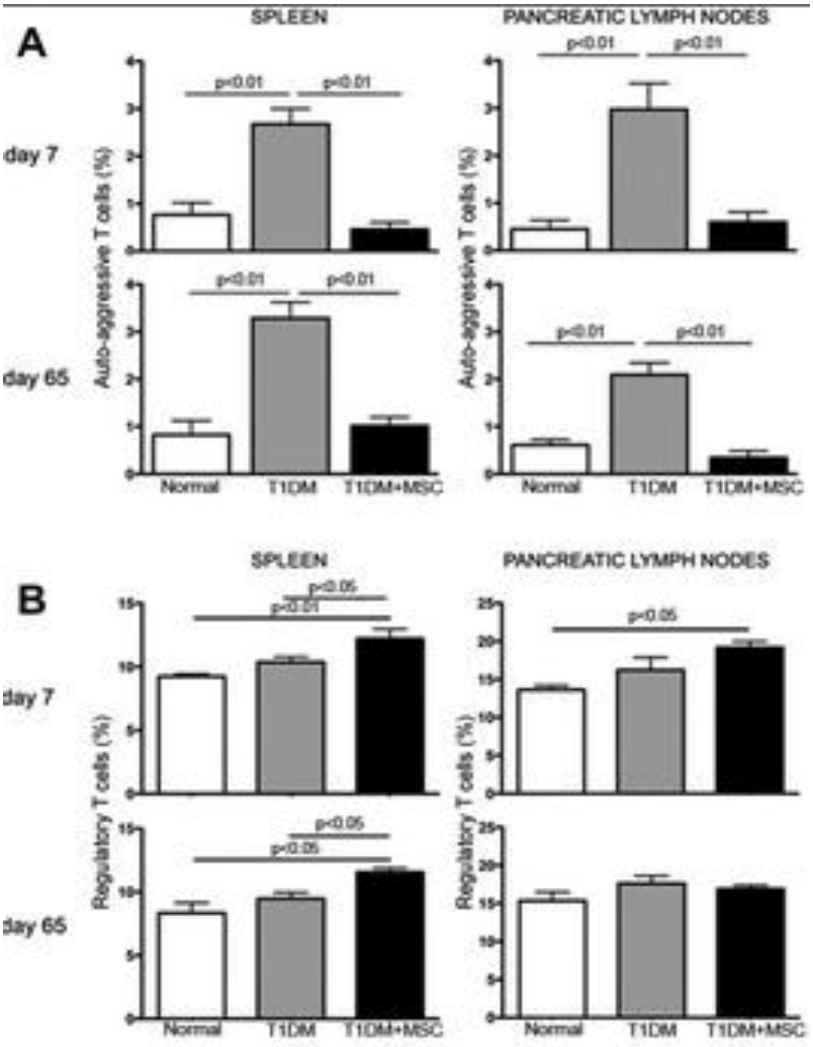


**Figure 8. MSCs restore the balance between regulatory and autoaggressive T cells:** (A) The autoaggressive T cell count is highest in mice with untreated T1DM, and diabetic mice given MSCs were shown to have near wildtype levels systemically, and lower than wildtype levels in the pancreas. (B) The amount of regulatory T cells is highest in mice given the MSC treatment.

As with MSCs, HSCs possess the capability to engraft in the host *in vivo*. Expression of the particular homeobox gene HoxB4 confers the ability to functionally engraft and thus contribute to the long-term restoration of depleted blood lineages. Successful expression of HoxB4 is associated with both the expansion of the HSC population *in vivo* as well as promotion of the self-renewing divisions of HSCs *in vitro*, and is needed to induce the expression of at least two biomarkers of definitive HSCs[36]. Upstream inducers of HoxB4 as well as the downstream targets that allow for the acquisition of hematopoietic functions of HSCs remains unknown and a target for current stem cell research. HSC mobilization and proliferation is often stimulated by treatment with particular cytotoxic drugs such as cyclophosphamide or hydroxyurea that are administered during the transplantation process[60].

A unique characteristic of HSCs is their lack of programmed death ligand 1 (PD-L1), which is an important immune checkpoint in the T1DM mouse model. PD-L1 plays an essential role in maintaining immune tolerance by controlling and inhibiting activated T cells. The cross play between the PD-L1 ligand and its receptor, expressed primarily on activated T cells, inhibits T cell activation and favors apoptosis, and thus mice deficient in PD-L1 develop diabetes at an accelerated rate[62]. A genome-wide assay revealed that a network of microRNAs exist that are in control of PD-L1 expression, and that silencing of one of the key altered microRNAs successfully restored PD-L1 expression in HSCs. Therefore, it was reasoned that restoration of the PD-L1 defect could serve as a cure for T1DM as

an alternative to an immunosuppressive regiment. To do so, HSCs were genetically modified to overexpress PD-L1 and transplanted into a mouse model. Results showed an inhibition of the autoimmune response *in vivo*, a reversion in the diabetic state in a newly hyperglycemic model, as well as homing of the HSCs to the pancreas. Human patients with T1DM were shown to have the same PD-L1 expression defect, and modified human HSCs also inhibited the immune response *in vivo*[7].

**II. Clinical Effectiveness of MSC Therapy**

The early 2000s provided a boom in the exploration of treatment options for T1DM. Islet and pancreatic transplantations were both being tested with promising normoglycemic results, but a lack of donors and complicated surgery needed for the procedure provided road blocks to major advancements. Concurrently, stem cell therapy was being explored in many fields of regenerative medicine. Progress was already seen in generating cardiac cells capable of repairing heart tissues and stem cells were also used to replace cells damaged by chemotherapy. In 2004 the first experiment was done demonstrating the differentiation capabilities of MSCs into functional islet-like cells in an *in vitro* animal model. Diabetes is induced in the mice via streptozotocin, a compound that is used clinically to treat pancreatic Beta-cell carcinoma, as streptozotocin attacks and damages Beta-cells. After around 2 weeks (depending on the dose), hypoinsulinemia and hyperglycemia result in the mice due to insufficient Beta-cell production of insulin[26].

The first major breakthrough in islet cell replacement for insulin-dependent diabetics was published in the year 2000, and was established as the Edmonton Protocol[53]. Shapiro and co-workers performed islet transplantation in 36 patients with T1DM in conjunction with an immunosuppressive regiment. All 36 patients received islet transplants from two or more donor pancreases and were able to quickly sustain decreased insulin dependence and had lowered need for exogenous insulin. A total of 21 subjects achieved insulin independence with proper glycemic control at any point in the trial, with 16 of these requiring insulin again at 2 years, and 5 remaining insulin independent. Some subjects experienced a decline in renal function, and all were subject to lifelong immunosuppressant medication[53]. Despite these shortcomings, the Edmonton Protocol established a single transplantation method, with higher yield and greater consistency than any prior. Proving the efficacy of islet-cell transplantation laid the foundation for differentiating MSCs into IPCs for the treatment of T1DM.

Therapeutic benefits resulting from the usage of MSCs is quantified on four major criteria: fasting plasma glucose levels, C-peptide levels, glycated hemoglobin, and daily insulin dose. Fasting plasma glucose measures one's blood sugar level after refraining from eating for at least 8 hours, and individuals with T1DM are expected to have higher levels than their healthy counterparts. Glycated hemoglobin refers to the hemoglobin (carrying oxygenated blood) that is chemically linked to glucose, and is generally higher in diabetics as a result of higher blood glucose levels. Daily insulin injection dose is the concentration of exogenous insulin

that is given each day. Combined, these measures are used to indicate the success of MSC treatment[13].

Initial experiments were done on rat models in order to determine if properly differentiating MSCs into IPCs could in turn lead to increased insulin production[12]. Femoral bone marrow derived stem cells were isolated from healthy rats and induced to differentiate into functional pancreatic cells following established protocols. A radioimmunoassay was applied on MSCs after isolation in order to assess insulin excretion level. The insulin mRNA excreted from a sample of differentiated MSCs was compared to that from a sample of undifferentiated MSCs, summarized in Table 2[12]. To test the glucose-controlling function of these differentiated MSCs, six diabetic rat models were selected. Each rat received a subcutaneous injection, with three rats receiving IPCs differentiated from MSCs, two receiving undifferentiated MSCs, and one not receiving any cells. The individual blood glucose levels of each rat was compared before the injection and one week after treatment, summarized in Table 3[12]. Through these experiments, Chen and his laboratory were able to successfully show the differentiation capabilities of MSCs to IPCs that were both morphologically similar to and played a similar physiologically role as endogenous pancreatic islet cells when transplanted into a mammalian model.

| Group | Insulin excretion in pre-treated MSCs | Insulin excretion in treated islet-like cells |
|---|---|---|
| 1 | 1.67 | 410.79 |
| 2 | 2.53 | 383.21 |
| 3 | 1.53 | 465.81 |
| 4 | 3.36 | 308.28 |
| 5 | 2.20 | 516.45 |
| 6 | 3.40 | 597.02 |

**Table 2. Insulin secretion changes in pre- and differentiated MSCS (IU/L):** MSCs require a proper transdifferentiation protocol into islet-like cells in order to produce significant amounts of insulin[12].

| Types of cells injected | Glucose level 24 h before injection | Glucose level 1 w after injection |
|---|---|---|
| Islet1 | > 33.3 | 25.4 |
| Islet2 | > 33.3 | 21.4 |
| Islet3 | 25.3 | 19.7 |
| MSC1 | > 33.3 | > 33.3 |
| MSC2 | 28.9 | 29.7 |
| Non | > 33.3 | > 33.3 |

**Table 3. Blood glucose levels for IPC and undifferentiated MSC treatment:** Rats given MSCs differentiated into IPCs (Islet1, Islet2, Islet3) showed decreases in blood glucose levels 1 week post-transplantation, whereas rats given undifferentiated MSCs (MSC1, MSC2), or no treatment (non), showed no reductions in blood glucose levels after 1 week[12].

Successful transdifferentiation protocols for MSCs produce cells capable of expressing the insulin protein and have physical characteristics resembling that of endogenous Beta-cells. Early trials saw the collection of IPCs that were functional *in vitro*, but failed to produce insulin when transplanted into a hyperglycemic model[54]. Human trials were put on hold until a proper transdifferentiation procedure that produced glucose-responsive IPCs *in vivo* was discovered. Consequently, the usage of MSCs in humans with T1DM is an even newer field, with the earliest trials starting in 2008. This study included only five patients, aged 14 to 28, whose blood glucose levels were not improving under their current insulin therapy[56]. After intraportal transplantation of a combination of AD-MSCs derived IPCs along with HSCs, all patients showed a 30% to 50% decrease in insulin requirements along with a 4 to 26-fold increase in C-peptide levels by the end of the study. Three of the patients had a history of diabetic ketosis episodes, and after receiving MSC transplants, these episodes appeared to go away. Throughout the five month follow up period, no patients experienced any unwanted side effects or required the use of any immunosuppression medications.

A similar trial involving eleven patients with T1DM receiving an intraportal administration of AD-MSCs that had been transdifferentiated to IPCs along with HSCs was published in 2010[58]. As with the Trivedi study[56] before it, pregnant women and anyone experiencing serious health complications resulting from diabetes were excluded from the study. A chemiluminescence assay showed that these cells secreted C-peptide *in vivo* in response to an increase in glucose

concentration. Patients were followed over a much longer period of time, 23 months. At the end of the study, all patients showed a mean decrease in exogenous insulin requirements as well as a mean increase in C-peptide levels. Further, treated patients on a normal diet and who performed normal physical exercise became free of diabetic ketoacidosis without weight gain and without any unwanted side effects, all without the usage of immunosuppressants. Although these patients still failed to achieve full insulin independence, the safety and effectiveness of AD-MSC based therapy in a longer term study were achieved.

In another clinical study, the long-term efficacy of Wharton jelly derived-MSCs (WJ-MSCs) was assessed in 15 patients diagnosed with T1DM over a 24-month period[30]. As with the prior studies, insulin requirements dropped and C-peptide levels increased significantly compared to the control group. Most notably, three patients had become completely insulin independent by the end of the follow-up period. This was the first reported case of achieving insulin independence in a clinical trial. No reports of any chronic of acute side effects, including ketoacidosis, were noted in the MSC group, while in the control group 3 patients continued to experience ketoacidosis.

Not all clinical trials have seen such ubiquitous benefits. A prospective randomized clinical study in which BM-MSCs were harvested from the iliac crest were intravenously transplanted in 20 patients with recent-onset T1DM. After 4 weeks post-infusion, C-peptide levels were increased, but this was only temporary. One year following the transplant, there was no significant increases in C-peptide

levels, glycated hemoglobin levels, or changes in required insulin dose. However, there were no apparent negative changes in any of these values, indicating that the diabetic condition did not worsen in any of the patients[9].

Recently, patients undergoing stem cell therapy have received their treatment in a 4 step method, summarized in Figure 9[13]. Initially, bone marrow is stimulated *in vivo* by colony stimulating factor, which induces the bone marrow to produce stem cells and release them into the bloodstream. Concurrently, bone marrow is extracted and implanted into the liver. Next, AD-MSCs (either autologous or allogenic) are differentiated into IPCs and implanted into the portal circulation. Then, a mix of MSC-derived IPCs as well as HSCs from the bone marrow are injected into portal circulation. Finally, BM-MSCs are intravenously infused into the patient.

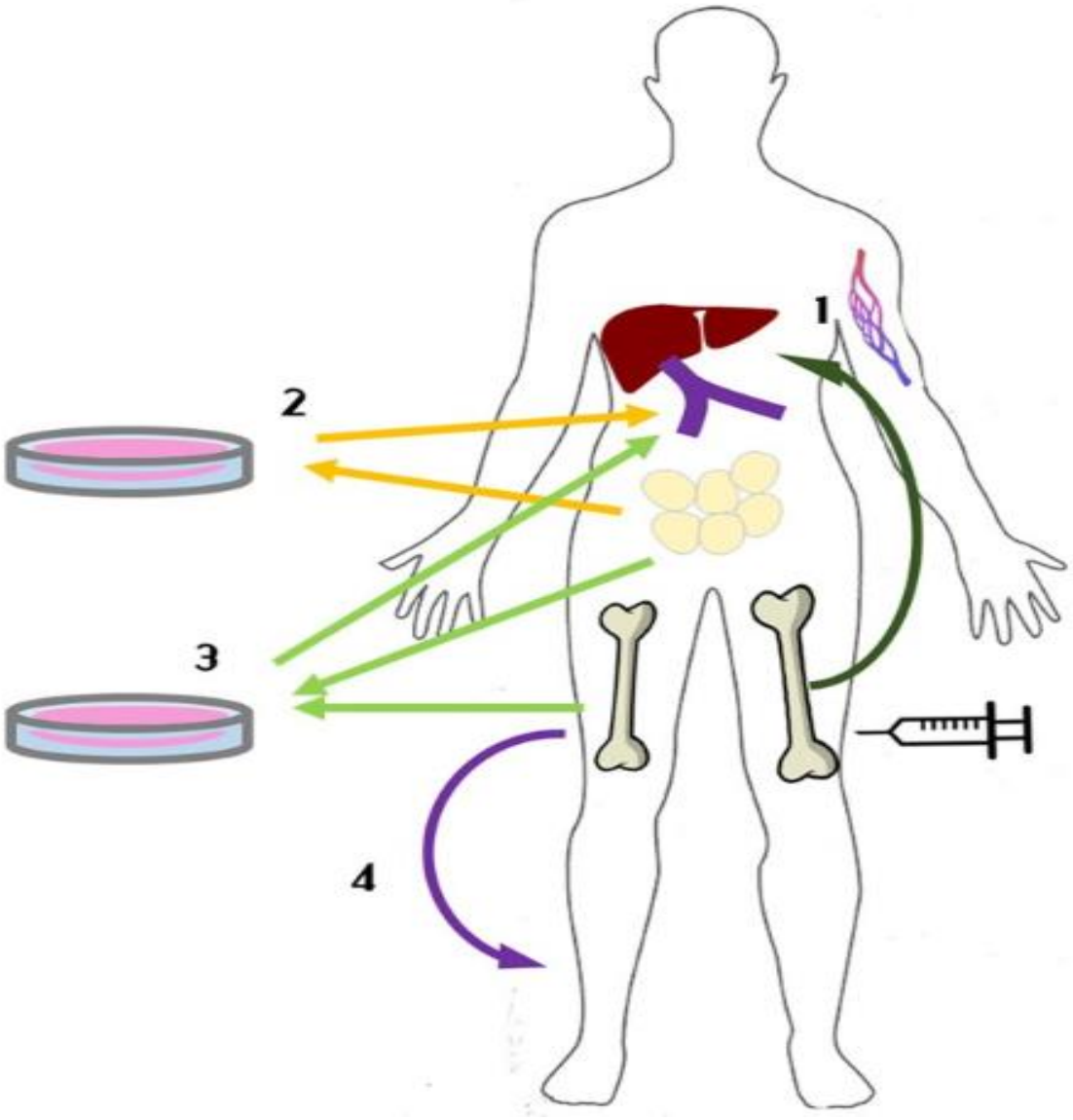


**Figure 9. Schematic of MSC transplantation in clinical trials:** 1) Autologous BM-MSCs are transplanted in patient if applicable. 2) AD-MSCs, either autologous or allogenic, are differentiated into IPCs and implanted in patient. 3) AD-MSC derived IPCs and HSCs from bone marrow are injected into patient. 4) BM-MSCs are infused intravenously[13].

A meta-analysis of individuals with T1DM who had received stem cell therapy showed that a fifth of patients showed adverse effects, which is still a safer form of transplantation therapy than either islet or whole pancreas transplantation[20]. However, these adverse effects should not be completely ignored. Most of the problems arose as a result of the administration of a high-dose of immunosuppressive regimen given before the transplantation. These side effects were alleviated shortly after the transplantation and subsequent immune reconstitution occurred. Still, lower doses of better tolerated immunosuppressive medications along with stronger prophylaxis against infection appears to be necessary to improve therapeutic outcome.

The first clinical trial involving the use of autologous HSC transplantation for individuals with T1DM, with the goal of exploring potential adverse side effects and evaluating potential applications of such therapy, occurred in 2014. 65 individuals with new-onset T1DM were enrolled in the study and followed up over a 48 month period. Within the first 6 months of receiving a single HSC infusion along with an immunosuppression therapy, 59% of patients were able to achieve insulin independence, with 32% of all patients remaining insulin independent by their last follow up. Every subject showed signs of improved glycemic management via decreased glycated hemoglobin and increased C-peptide levels compared with their pretreatment baseline measures. Although the immune system of the patients showed near complete recovery, such as leukocyte count, over half of the treated individuals experienced some sort of adverse effect. A severe infection was noted in

3 of the individuals, with 1 subject dying as a result of sepsis and disseminated intravascular coagulation, a condition in which the proteins that control blood clotting become overactive[16]. A list of all of the adverse events that the patients experienced during the treatment and follow up is presented in table 4.

| | n |
|---|---|
| Neutropenic fever | 7 |
| Alopecia, gastric tract symptoms | 5 |
| Alopecia, fever | 3 |
| Fever, bone marrow suppression | 2 |
| Allergic reaction to ATG | 2 |
| Patient died due to sepsis and DIC | 1 |
| *Staphylococcus haemolyticus* bacteremia | 1 |
| Nausea, fever, rash due to ATG | 1 |
| Neutropenic fever, mucositis, coagulopathy | 1 |
| Transient upper right limb edema during conditioning (no signs of thrombosis) | 1 |
| Pneumothorax after CVC insertion | 1 |
| Skin rash, neutropenic fever | 1 |
| Gastric tract symptoms, fever | 1 |
| Alopecia, fever, failure of stem cell harvest | 1 |
| *Pseudomonas aeruginosa* sepsis | 1 |
| Alopecia, gastric tract symptoms, nutrition support | 1 |
| Alopecia, fever, hematuria | 1 |
| Gastric tract symptoms, fever, bone marrow suppression | 1 |
| Gastric tract symptoms, granulocyte suppression | 1 |
| Diarrhea | 1 |
| Total number of subjects experiencing adverse effects | 34/65 |

**Table 4. Unfavorable side effects experienced during HSC treatment:** Of the 65 patients enrolled in the D'Addio T1DM study utilizing HSCs as treatment, 34 experienced undesired side effects. ATG, antithymocyte globulin; CVC, central venous catheter; DIC, disseminated intravascular coagulation[16]

# DISCUSSION

## Discussion

The clinical benefit of MSCs is certainly evident, not just in the field of diabetes, but in the entire field of regenerative medicine. Expectations must be tempered, however, as stem cells cannot simply be used as a one trick fix for the direct replacement of dysfunctioning organs. Instead, it is important to understand the physiology behind which such cells exhibit their therapeutic benefits in order to best apply them in the clinic. By specifically discerning how MSCs are isolated and can transdifferentiate into IPCs to be transplanted into humans for glycemic control, scientists can better grasp how these cells work and how they can be better used to treat patients. Understanding the physiological basis behind their immunomodulatory properties allow for more precise experiments involving MSCs and can lead to novel breakthroughs.

A key finding in these clinical studies is the apparent decrease in amount of exogenous insulin that is required for proper glycemic control when given a single transplant of insulin producing MSCs. Yet, only a small minority of patients in all of these studies were able to achieve and maintain complete insulin independence by the end of the trial time period. This may result from inadequate dosing procedures caused by lack of current data, or the need for multiple MSC transplants given at different time points. To date, there have not been any experiments considering multiple intravenous transplants of MSCs into humans, but such data is available for mouse models. In such a study, human MSC transplants were given every 2 weeks

for a 6-month period. Compared to the mice receiving a single transplant, who saw transient decreases in blood sugar levels that lasted for around a month, the mice given multiple injections saw effective restoration of glucose homeostasis. Systemic oxidative stress was decreased after the 7th week, and production of human insulin was dramatically increased and stabilized starting in the 11th week as MSCs engrafted and subsequently differentiated into IPCs[29]. Unfortunately for individuals who are unresponsive to a first MSC transplantation, repeated administration has shown no physiological advantages[28]. For those who experience improved glycemic control when given an initial MSC transplant, perhaps giving multiple doses over a duration of time could prove to be more successful in alleviating the need for a constant supply of exogenous insulin.

Perhaps a combination therapy method may be more effective than MSC transplantation alone. Combination therapy has certainly proved more effective than monotherapy in the treatment of diseases such as chronic obstructive pulmonary disease, cancer, and HIV/AIDS, so it is reasonable to delve into whether this is a feasible option for T1DM. A few of the studies discussed here included HSC transplantation concurrently with MSCs, as bone marrow derived HSCs have showed similar outcomes as MSCs in improving C-peptide levels and decreasing glycated hemoglobin levels. Still, these data are murky and lack conclusiveness. The one large study on T1DM conducted utilizing solely HSCs as the source of stem cells showed extremely variable results. Even though around a third of the subjects achieved and maintained insulin independence by the end of the two-year study, 34

of the 65 of individuals experienced negative side effects. Worse yet, one patient died as a result of infection by *Pseudomonas aeruginosa* leading to sepsis. These data suggest much more research needs to be done on the efficacy and safety of HSC transplantation. With diabetes disrupting the microenvironment of the bone marrow leading to abnormalities in endogenous HSCs, it appears valid that some kind of HSC replacement could be an effective means of combating some of the symptoms of the disease. Since HSCs play a major role in remodeling the diabetic microenvironment in a pro-insulin secreting way, this would appear to greatly complement the MSCs effect of improving Beta-cell efficiency. Thus, a multifactorial approach to T1DM may be the most suitable method to achieving long-term glucose independence while minimizing any sort of adverse side effects.

As diabetes has become more prevalent across the developing world, there has become an increasing need to find novel therapeutics to combat the issue rather than simply manage it. This thesis focused on the advances within the field of stem cell transplantation, but progress is also being made in other treatment options. As discussed, islet cell replacement therapy is a viable option for insulin dependent diabetics.

A California company is currently developing islet-cell replacement therapies that may prove capable of overcoming the need for lifelong immunosuprresants and glucose monitoring as well as the limited number of donor cells that riddle classical islet cell transplantations. Viacyte's PEC-Direct and PEC-Encap both transplant lab-created human pancreatic progenitor cells into patients, where the cells are able to

mature into functional pancreatic islet cells that induces insulin-producing Beta-cells in response to rising glucose levels. These products are surgically implanted under the skin and incorporate an encapsulated device design that protects the cells from the host's immune system. However, the current evidence is limited to phase I and II human trials that evaluate the safety, as there is yet to be sufficient data about efficacy in humans, and the length of time that the product can safely and functionally remain implanted is yet to be determined. There is also no known estimation about the future cost of ViaCyt's products or what the cost to the consumer would be[15]. Regardless, ViaCyt is taking advantage of some of the properties of stem cells such as immune evasion and ability to produce large amounts of cells in a lab using PCR in order to try and combat diabetes in a new and promising method. The results of their ongoing phase I and II trials could show how proper ingenuity and creative modification of a current treatment option's shortcomings can lead to better treatment.

The ability to hide cells from the host's immune system via an encapsulation method is an exciting advancement in improving the transplantation process. Usage of an encapsulation method with AD-MSC derived IPCs has been shown to promote normoglycemia in diabetic mice after 2 weeks when transplanted into the peritoneal cavity[11]. The transplanted capsules themselves were removed after 4 weeks, with the IPCs retaining their cellular integrity and function. In another study, BM-MSCs were differentiated into IPCs and encapsulated and transplanted in a similar method, and a second group of diabetic mice received a transplant with

unencapsulated IPCs via the intraportal route[45]. They demonstrated that IPCs in the mice that received the encapsulated transplant had greater survival than the unencapsulated group, and the mice had further reductions in immune response while having similar improvement in hyperglycemia. Cotransplantation of MSCs and encapsulated MSCs showed an improvement in the oxygenation of the encapsulated cells due to an increase in vascularization of the pancreas, yet more studies need to be done before this bidimensional approach is applied in human therapy. Should these studies prove successful, this would be another small step forward in improving the efficacy of stem cell treatment in the treatment of diabetes.

A key focus of this thesis is to highlight the inadequacy of the current available data on stem cell treatment and the importance of further exploration. This work primarily rests on the shoulders of research scientists performing quantitative laboratory experiments that can provide statistically significant results. Yet, it is not the sole responsibility of researchers to further the field. Both doctors and patients should be more open to the idea of alternative therapies for T1DM when exogenous insulin is not adequate in managing the disease. One of the major hold ups in the clinical trials discussed was patient recruitment. As stem cell transplantation is such a new field of medicine, it is often difficult to recruit patients to participate in these studies, and there is no easy way to convince them with the current lack of conclusive evidence. To combat this issue, it's important for physicians to be open to recommending these sorts of novel therapies and be

diligent in describing their potential benefits to patients. Reminding them that their blood glucose levels will still be constantly monitored throughout the duration of the trial, and that exogenous insulin will still be given when needed could alleviate some of the fears that patients may have.

Certainly, the studies to date have demonstrated that stem cell transplantation can be considered a safe and effective alternative treatment for many individuals with T1DM. Yet, many of the cases considered in this manuscript have been evaluated solely in a single study, often with low statistical power. The few patients enrolled in each study, often with a relatively short follow-up time, may not have been enough to allow for significant statistical interpretation. Also, there is great heterogeneity in the makeup of the samples between studies. For example, the Carlsson study used patients who were recently diagnosed with T1DM, while the Vanikar and Trivedi studies included patients who had been previously diagnosed with T1DM for anywhere from 1 to 20 years[9]. Increasing the number of trials, along with the number of patients in each, for a longer duration of time may allow for powerful conclusions to be drawn. These shortcomings are not necessarily reflective of MSCs as a treatment option, but rather reflect the insufficiency of available published data on the topic. Much research still needs to be done on the specific interactions between stem cells and endogenous islet cells, and the specific pathways that lead to the clinical benefits that MSCs are able to exhibit in patients.

The results of these studies indicate that MSCs have the potential to play a serious role in combating the etiology of T1DM. Though their mechanisms of action

are not completely understood yet and they are not completely effective in ridding patients of the need for exogenous insulin, MSCs have shown the ability to alleviate some of the symptoms of diabetes. Larger scale studies involving greater sample sizes carried out over a longer duration of time still need to be conducted. Future studies should be undertaken involving HSC supplementation along with encapsulation methods in order to try and maximize the benefits of MSCs *in vivo*. Although promising, MSC transplantation is still far away from becoming a permanent cure for T1DM, and any patient considering this treatment option should be aware of potential side effects and remain on a constantly monitored insulin routine.

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**VITA**